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Method for the determination of ammonium in cigarette tobacco using ion chromatography

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Abstract

Ammonia and other alkaline substances have been postulated to be important in cigarette design. The most significant potential contribution of ammonia is a possible interaction with the native, protonated nicotine in the smoke. Ammonia is more alkaline than nicotine and could facilitate a shift in the acid/base equilibrium where a fraction of the total nicotine converts to the more lipophilic, non-protonated form. This non-protonated, or free-base, form of nicotine absorbs more efficiently across membranes, resulting in more rapid delivery to the smoker's bloodstream. Ammonia and other potential ammonia sources, such as additives like diammonium phosphate, could influence the acid–base dynamics in cigarette smoke and ultimately the rate of nicotine delivery. To examine and characterize the ammonia content in modern cigarettes, we developed a fast, simple and reliable ion chromatography based method to measure extractable ammonia levels in cigarette filler. This approach has minimal sample preparation and short run times to achieve high sample throughput. We quantified ammonia levels in tobacco filler from 34 non-mentholated cigarette brands from 3 manufacturers to examine the ranges found across a convenience sampling of popular, commercially available domestic brands and present figures of analytical merit here. Ammonia levels ranged from approximately 0.9 to 2.4 mg per gram of cigarette filler between brands and statistically significance differences were observed between brands and manufacturers. Our findings suggest that ammonia levels vary by brand and manufacturer; thus in domestic cigarettes ammonia could be considered a significant design feature because of the potential influence on smoke chemistry.

Keywords

Ammonia; Cigarettes; Ion chromatography

1. Introduction

Ammonia or compounds serving as potential ammonia precursors, such as diammonium phosphate (DAP) and urea are commonly used in manufacture of the reconstituted tobacco

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sheet (recon) or can be added directly to cigarette filler depending on the product (Johnson, 1984; Christopher, 1978; Unknown, 0000; Ammonia Disposition in Marlboro, 0000). The U.S. Food and Drug Administration (FDA) has argued that ammonia chemistry plays an important function increasing and controlling the nicotine delivery to smokers by raising the “effective” pH of tobacco smoke which works to increase nicotine’s bioavailability (Administration, 1995). In support of this argument, FDA quoted in its 1995 *Federal Register* Brown & Williamson’s (BW) 1991 Handbook on Leaf Blending and Product Development: “Ammonia, when added to a tobacco blend, reacts with the indigenous nicotine salts and liberates free [-base] nicotine. As a result of such change, the ratio of extractable nicotine to bound nicotine in the smoke may be altered in favor of ... [free-base] nicotine. As we know, extractable (i.e., free-base) nicotine contributes to the impact of cigarette smoke; by increasing free base form, ammonia can act as an impact booster” (Administration, 1995). Compared with the non-volatile, protonated form of nicotine, which is hydrophilic, free-base nicotine is lipophilic, volatile, and rapidly absorbs across membranes into a smoker’s bloodstream, contributing to the “impact” of the cigarette (Wayne and Carpenter, 2009; Henningfield et al., 2004; Armitage and Turner, 1970; Schievelbein et al., 1973a,b).

The adoption of ammonia technology in the cigarette industry resulted in a flurry of competitive analyses among the top manufacturers. In the early 1960s, Philip Morris (PM) was the smallest of America’s then six leading cigarette companies, and RJ Reynolds’s (RJR) brand Winston experienced annual sales about three times those of PM’s Marlboro (Bates et al., 1999). By 1978, however, a huge shift in sales had occurred, with Marlboro accounting for one in five of all cigarettes sold (Bates et al., 1999). This shift prompted intense research efforts by the other tobacco manufacturers, and after reverse engineering of Marlboro cigarettes, it was concluded that ammonia technology was the key factor in the success of PM sales (Freedman, 1995). An internal study performed by RJR concluded that Marlboro sales during 1965–1974 were directly proportional to an increase in cigarette smoke pH, which led to an increase in free-nicotine content in the smoke as a result of PM’s use of ammonia technology (Teague et al., 1974).

In addition to its alleged role in converting a fraction of the total nicotine to the free-base form, introduction of ammonia-containing compounds during cigarette manufacturing may affect the organoleptic and mechanical properties of the tobacco. Ammonia can facilitate the formation of naturally occurring flavor compounds through various Maillard reactions (Francis et al., 1984; Wigand, 2006; Reynolds, 1992). For example, ammonia compounds such as ammonium hydroxide, DAP, diammonium citrate, and urea, are known to react with naturally occurring or added sugars to increase the levels of chemicals such as deoxyfructosazines, pyrazines, furans, and pyrroles (Johnson, 1989; Tang et al., 1991; Ihrig, 1973). These Maillard reaction products may contribute to the pleasant aromas of tobacco smoke and have been referred to as the “key of the cigarette chemistry” (Reynolds, 1992). In addition, Maillard reaction products may reduce the harshness perceived by the smokers when free nicotine levels are increased, allowing for a greater “impact” with reduced harshness (Christopher, 1978; Crouse, 1980).

Based on reports suggesting that ammonia may influence the rate of nicotine uptake, we developed a quantitative method to analyze the range of extractable ammonia content in cigarette tobacco filler using ion chromatography (IC). This method requires minimal sample preparation, has high throughput and has sufficient accuracy and precision to measure ammonia in domestic cigarette products. To apply the method, we examined ammonia levels in tobacco filler from 34 modern, popular-selling, domestic non-mentholated cigarette brands in the US. Ammonia levels were compared across manufacturers by cigarette brand and brand variant (brands with the same name but different machine smoked tar and nicotine deliveries) to see if statistically significant differences exist in the ammonia content of cigarettes formerly marketed as full-flavored, light and ultra-light. We have limited knowledge of how ammonia or ammonia precursors are added to tobacco products because such ingredients are often classified as “trade secrets.” Our goal was to analyze extractable ammonia in the cigarette filler and characterize variations as a function of manufacturer or brand.

2. Experimental

2.1. Sample collection and storage

Cigarette cartons were purchased locally and stored at room temperature prior to analysis. Before analysis, cigarettes packs were conditioned for 48 h at 22 °C and 60% humidity. For this analysis, cigarette packs remained unopened during conditioning due to internal findings indicating a substantial loss of ammonia (up to 30% difference) after 48 h of conditioning with an open cigarette pack (Fig. 1). Our data show that brands with a higher ammonia content decay more rapidly than those with lower initial ammonia content.

2.2. Materials

An ISO Guide 34 endorsed 1000 mg/L ammonium standard was purchased from Sigma Aldrich (St. Louis, MO, USA). Extraction bottles, caps, and sample vials were purchased from Lab Depot (Dawsonville, GA, USA). The ICS-3000 analytical system, columns, and methanesulfonic acid (MSA) cartridge used in the eluent generator were purchased from Thermo Scientific (Sunnyvale, CA, USA). Volumetric flasks were purchased from Thermo Fisher (Waltham, MA, USA). The deionized (DI) water used in this procedure is from an Aqua Solutions DI water system (Aqua Solutions, Jasper, GA).

2.3. Instrumentation and data analysis

Samples were run on a Dionex ICS-3000 analytical system controlled by Chromeleon version 6.8 software (Thermo Scientific, Sunnyvale, CA, USA). 25 µL of sample was injected onto a 4 mm Ion Pac CS12A cation exchange column (Thermo Scientific, Sunnyvale, CA, USA). Isocratic separation of the ammonium ion was achieved using a 20 mM methanesulfonic acid (MSA) eluent prepared by the EG40 eluent generator (Thermo Scientific, Sunnyvale, CA) that required only a deionized water source. The column temperature was maintained at 25 °C with a run time of 15 min. The pumps and eluent generators were turned on at least 60 min before the first injection to allow baseline stabilization. Ammonium ions were detected using a conductivity detector. Prior to detection by the conductivity detector, the MSA eluent conductance was suppressed by the cation self-

regenerating suppressor (CSRS). All ammonium values recorded in Chromeleon were transferred to Microsoft Excel 2010 for further analysis. Statistical evaluations were done using JMP Software (SAS Institute Inc., Cary NC, USA).

2.4. Sample preparation

All tobacco filler from individual cigarettes were removed from the paper wrapper and filter plug, placed into a 60-mL amber bottle and weighed. Deionized water (30 mL) was added and the sample was sealed with a PTFE-lined cap. The sample was shaken for 30 min at 160 rpm on a Barnstead/Labline Max Q 2000 shaker (Dubuque, IA, USA). A 1.5 mL sample aliquot was centrifuged in a Sorval Pico Biofuge from Thermo Fisher (Waltham, MA, USA) at 13,000 rpm for 5 min to remove tobacco debris. A 10:1 water to sample dilution was made in a 1.5 mL sample vial, vortexed and placed in a sample tray for analysis. Seven replicates were analyzed for each brand.

2.5. Calibration

Calibration standards were made up in 25 mL volumetric flasks with deionized water and ten serial dilutions of the 1000 mg/L ammonium standard. The calibration range for this study was 1– 10 mg/L, which was sufficient for all brands analyzed. The concentration range could easily be extended if the need arose with future tobacco products. A typical analytical batch included a blank, 2 quality control samples, calibration standards and up to 50 unknown samples. For sample quantification, a 10-point standard curve was run daily. The calibration curves were consistent with the combined quadratic regression (Seeman, 2007) yielding an $R^2 > 0.99$. The limit of detection (LOD), calculated using the Taylor method (Taylor, 1987), was 0.087 mg/L. The limit of quantification (LOQ) was 0.29 mg/L. The 3R4F research cigarette (University of Kentucky, Lexington, KY, USA) was selected as the QC material for the ammonia IC method and was included in each run to ensure system integrity and reproducibility.

3. Results and discussion

3.1. Development of chromatographic method

Isocratic separation for all common cation peaks (Li^+ , Na^+ , NH_4^+ , K^+ , Mg^+ , Ca^+) was achieved with baseline resolution of the ammonium and other peaks at 20 mM mobile phase concentration (Fig. 1). Previously reported methods for measuring ammonia in cigarette material use a variety of extraction solutions. Because ammonia and most simple ammonium salts are highly water soluble, DI water was used to extract ammonia from tobacco filler. We examined other extraction solvents including dilute sulfuric (Watson, 2015) and dilute acetic acid (Counts et al., 2006) solutions alongside deionized (DI) water. A popular American blend cigarette with a high level of extractable ammonia was selected for the comparison, and we found no significant differences between the water, sulfuric acid and acetic acid extractions. (Table 1) The ICS-3000 is a dual-system ion chromatograph that can simultaneously analyze cations and anions from the same sample. The relatively high amount of sulfate and acetate anions from the acid extraction solutions caused carryover issues in the system designated for anion analysis. Using DI water as an extraction solution eliminates the above carryover issues and acid waste. It should be noted, however, that the

sulfuric acid extraction solution did give slightly higher detector response, which could be useful for low-ammonia matrices.

3.2. Accuracy and precision

We examined sample recovery using a low-ammonia content commercial cigarette that was spiked with a known amount of ammonium chloride and method protocol for sample preparation was followed for these and corresponding unspiked samples. The average recovery was 82%. The analytical system recovery was examined by spiking a known amount of ammonium chloride into DI water and following method protocol for sample preparation; the recovery was 98%. For this study, method precision was evaluated by calculating the relative standard deviation (RSD) of the 3R4F QC pool. Inter-day precision was 13.9% while intra-day precision was 5.3%. Direct comparisons to other reported ammonia values are limited because brand names are typically not reported. However, the 3R4F (previous formulations were designated 2R4F and 1R4F) research cigarette is commonly used and reported and can be compared across methods (Table 2). Our 3R4F values were consistent with other's reported values, when considering results are reported from different formulations over nearly a decade.

3.3. Ruggedness

A range of analytical conditions were examined to test for changes in results as a consequence of variations in the method. Conditions such as extraction solution volume (15–50 mL), extraction time (15–60 min), dilution factor (1:5–1:10), centrifuge time (2–10 min) and mobile phase concentration (15–25 mM) were systematically varied and examined. Only minor changes were observed under all modified conditions. Additionally, grinding the tobacco prior to analysis was explored, but no different results were observed between ground and unground tobacco samples. While the method itself is extremely rugged, results are sensitive to storage conditions, i.e., opening the packs prior to analysis. Based on data examining the effects on ammonia content loss over time (Fig. 2), we recommend not opening packs until the time of analysis.

3.4. Ammonia levels in tobacco filler

The ammonia IC method provides quick and precise measurements of ammonia in cigarette tobacco filler. While not shown, this method is applicable to other tobacco products such as smokeless, loose leaf, roll-your-own, and pipe tobaccos. For this study, we selected 34 non-mentholated, American blended, commercially available brands for analysis. Ammonia was detected in all brands analyzed well above LOD. The average ammonia levels (from seven replicate measurements) in the tobacco filler from all cigarette brands ranged from approximately 0.87 to 2.41 mg ammonia per gram of conditioned tobacco filler (Fig. 3). Within-brand replicate measurements had relative standard deviations ranging from 2.7% to 12.3% ($n = 7$). Statistically significant differences ($p < 0.05$) were observed when comparing brands from different manufacturers; these differences can be visually observed in Fig. 4. The average ammonia (mg ammonia/g tobacco) levels in the tobacco filler were highest for manufacturer A followed by manufacturers B and C, respectively (Fig. 4). We found no statistically significant difference in average ammonia content among cigarettes categorized as having high, medium and low machine smoked delivery for tar and nicotine. No

correlations with ammonia content were found when other parameters such as rod length, tobacco weight, and measured tar, nicotine, water and CO values were evaluated.

4. Conclusion

We developed a rapid and robust analytical approach for quantitatively measuring ammonia extracted from cigarette filler material using an IC based technique. The IC method has sufficient sensitivity, reliability, and throughput for routine ammonia analysis in cigarette filler. The key findings of the current work are that ammonia levels differ in select brands and that the levels are manufacturer dependent. These differences presumably reflect the different design philosophies of cigarette manufacturers. Currently, the role of ammonia technology in cigarette design is unknown and widely debated, (Henningfield et al., 2004; Seeman, 2007; Lauterbach, 2010; Ashley et al., 2009; Watson et al., 2004; Seeman and Carchman, 2008; Dixon et al., 2000) however a lengthy discussion on the fate of ammonia from filler to smoke as well as its role in free nicotine delivery is beyond the scope of this work but can be found here (Watson, 2015). The role of ammonia in cigarettes and other tobacco products may not be completely understood, but the amount of industry interest over the past decades certainly suggest that ammonia plays an important role in cigarette design, which could have implications related to consumer satisfaction.

References

- Administration, F. a. D; Services, H. a. H, editor. Analysis Regarding The Food and Drug Administration's Jurisdiction Over Nicotine-Containing Cigarettes and Smokeless Tobacco Products. 1995; 60:41453–41787.
- Ammonia Disposition in Marlboro. Bates: 599000153/0180. <http://legacy.library.ucsf.edu/tid/bya30f00>
- Armitage AK, Turner DM. Absorption of nicotine in cigarette and cigar smoke through the oral mucosa. *Nature*. 1970; 226(5252):1231–1232. [PubMed: 5422597]
- Ashley DL, Pankow JF, Tavakoli AD, Watson CH. Approaches, challenges, and experience in assessing free nicotine. *Handb Exp Pharmacol*. 2009; 192:437– 456.
- Baker RR, Pereira da Silva JR, Smith G. The effect of tobacco ingredients on smoke chemistry. Part I: Flavourings and additives. *Food Chem Toxicol*. 2004; 42(Suppl):3–37.
- Bates C, Connolly GN, Jarvis M ASH, Fund ICR. Tobacco Additives: Cigarette Engineering and Nicotine Addiction: a Survey of the Additive Technology Used by Cigarette Manufacturers to Enhance the Appeal and Addictive Nature of Their Product. ASH. 1999
- Christopher, FH, Jr. [Accessed October 9, 1978] Free nicotine/ammonia treatment of tobacco. 1978. Bates: 505197081–7081 <http://legacylibrary.ucsf.edu/tid/cru46b00>
- Counts ME, Morton MJ, Laffoon SW, Cox RH, Lipowicz PJ. Smoke composition and predicting relationships for international commercial cigarettes smoked with three machine-smoking conditions. *Regul Toxicol Pharmacol: RTP*. 2005; 41(3):185–227. [PubMed: 15748796]
- Counts ME, Hsu FS, Tewes FJ. Development of a commercial cigarette “market map” comparison methodology for evaluating new or non-conventional cigarettes. *Regul Toxicol Pharmacol: RTP*. 2006; 46(3):225–242. [PubMed: 16989926]
- Crouse, WE. The Effect of Adding Free and Bound Nicotine Alkaloid on the Physical/ Chemical and Organoleptic Properties of BGU-79 Tobacco 1980. Bates: 81022803–81022807. <http://legacy.library.ucsf.edu/tid/zkr46b00>
- Dixon M, Lambing K, Seeman J. I, Mini-review: On the transfer of nicotine from tobacco to the smoker. A brief review of ammonia and “pH” factors. *Beiträge zur Tabakforschung Int*. 2000; 19(2):103–113.

- Francis, S., Hsu, RS. Analytical Sensory Correlations: Liquid Ammonia Treated Tobacco. 1984. Bates: 2056130286/0304. <http://legacy.library.ucsf.edu/tid/kbr72e00>
- Freedman AM. 'Impact Booster' tobacco firm shows how ammonia spurs delivery of nicotine. Wall Street J. 1995
- Henningfield J, Pankow J, Garrett B. Ammonia and other chemical base tobacco additives and cigarette nicotine delivery: issues and research needs. Nicotine Tobacco Res. 2004; 6(2):199–205.
- Ihrig, AM. pH of Particulate Phase 1973. Bates: 87644270–87644281. <http://legacy.library.ucsf.edu/tid/iwr46b00>
- Johnson, R. The Unique Differences of Phillip Morris Cigarette Brands – R&D-B016-84. 1984. Bates: 103281081–103281112. <http://legacy.library.ucsf.edu/tid/ton66b00>
- Johnson, R. Ammonia Technology Conference Minutes. 1989. Bates: 508104012- 508104164. <http://legacy.library.ucsf.edu/tid/cfl36b00>
- Lauterbach JH. Comment on gas/particle partitioning of two acid-base active compounds in mainstream tobacco smoke nicotine and ammonia. J Agric Food Chem. 2010; 58(16):9287–9288. [PubMed: 20677754]
- Reynolds, R. Regarding Means to Achieve Nicotine Balance and Deliveries. 1992. Bates: 508408649–508408770. <http://legacy.library.ucsf.edu/tid/ikv46b00>
- Schievelbein H, Eberhardt R, Loschenkohl K, Rahlfs V, Bedall FK. Absorption of nicotine through the oral mucosa. I Measurement of nicotine concentration in the blood after application of nicotine and total particulate matter. Agents Actions. 1973a; 3(4):254–258. [PubMed: 4776369]
- Schievelbein H, Eberhardt R, Rahlfs V, Bedall FK. Absorption of nicotine through the oral mucosa. II Measurement of blood pressure after application of nicotine and total particulate matter. Agents Actions. 1973b; 3(4):259–264. [PubMed: 4776370]
- Seeman JI. Possible role of ammonia on the deposition, retention, and absorption of nicotine in humans while smoking. Chem Res Toxicol. 2007; 20(3):326–343. [PubMed: 17316028]
- Seeman JI, Carchman RA. The possible role of ammonia toxicity on the exposure, deposition, retention, and the bioavailability of nicotine during smoking. Food Chem Toxicol. 2008; 46(6): 1863–1881. [PubMed: 18450355]
- Tang, JY., MTP. DAP-Free Recon Development Update. 1991. Bates: 508102219/2224. <http://legacy.library.ucsf.edu/tid/dtm51f00>
- Taylor, JK. Quality Assurance of Chemical Measurements. Lewis Publishers; Chelsea, Michigan: 1987.
- Teague, CE. Implications and Activities Arising from Correlation of Smoke Ph with Nicotine Impact, Other Smoke Qualities, and Cigarette Sales. 1974. Bates: 509314122–509314144, 511223484. http://tobaccodocuments.org/product_design/344.html
- History of Ammonia Technology. Unknown Bates: 599003683/3690. <http://legacy.library.ucsf.edu/tid/iyq20f00>
- Watson CV. Role of ammonia in delivery of free nicotine: recent work and analytical challenges. WHO Technical Report Series. 2015; 989:163–174.
- Watson CH, Trommel JS, Ashley DL. Solid-phase microextraction-based approach to determine free-base nicotine in trapped mainstream cigarette smoke total particulate matter. J Agric Food Chem. 2004; 52(24):7240–7245. [PubMed: 15563201]
- Wayne GF, Carpenter CM. Tobacco industry manipulation of nicotine dosing. Handb Exp Pharmacol. 2009; 192:457–485.
- Wigand, JS. Additives, Cigarette Design and Tobacco Product Regulation. A Report to World Health Organization Tobacco Free Initiative Tobacco Product Regulation Group. 2006. Bates: 3990512671/3990512715 <http://legacy.library.ucsf.edu/tid/ccj13j00>

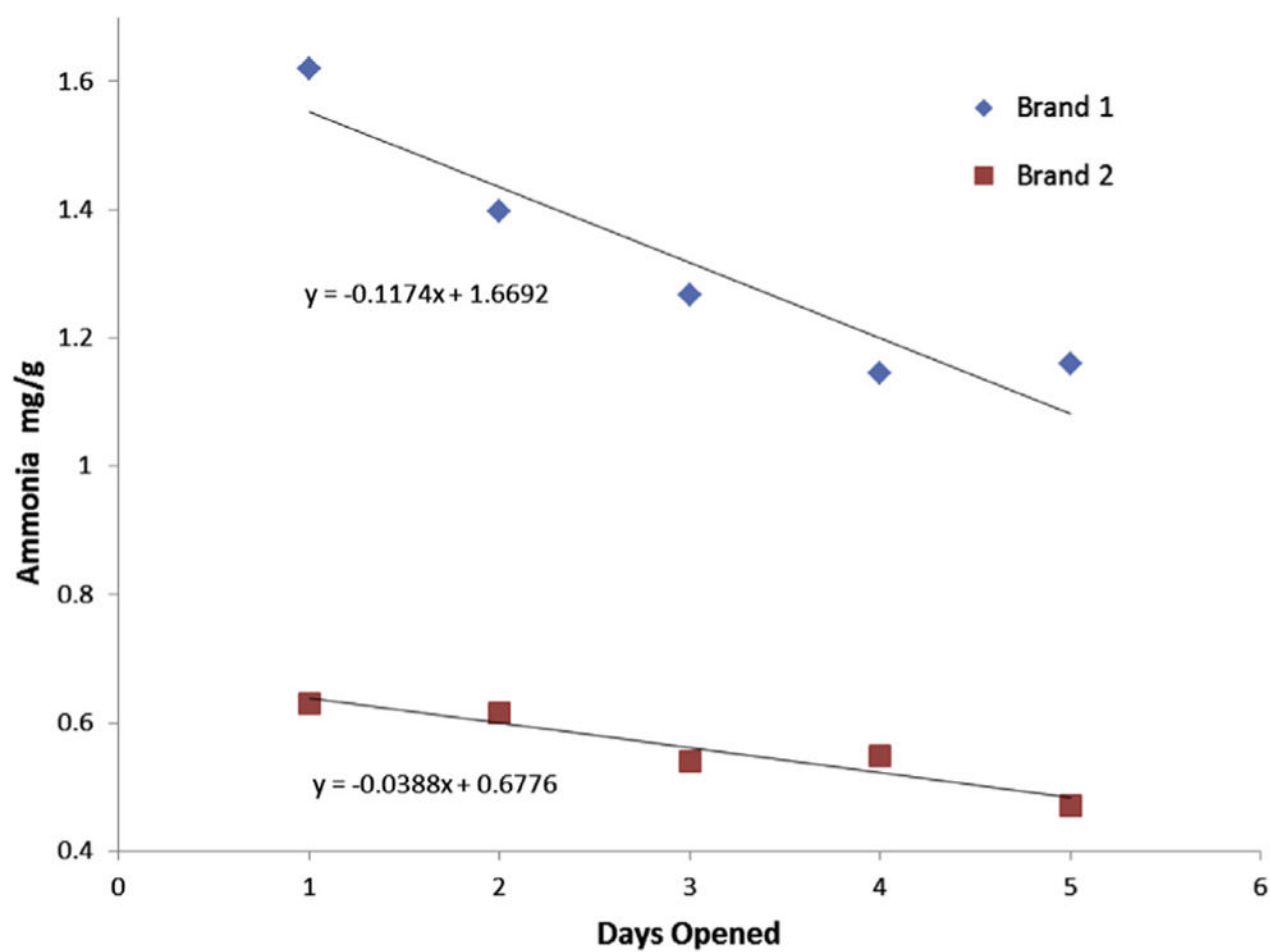


Fig. 1.
Chromatogram of the 3R4F reference cigarette.

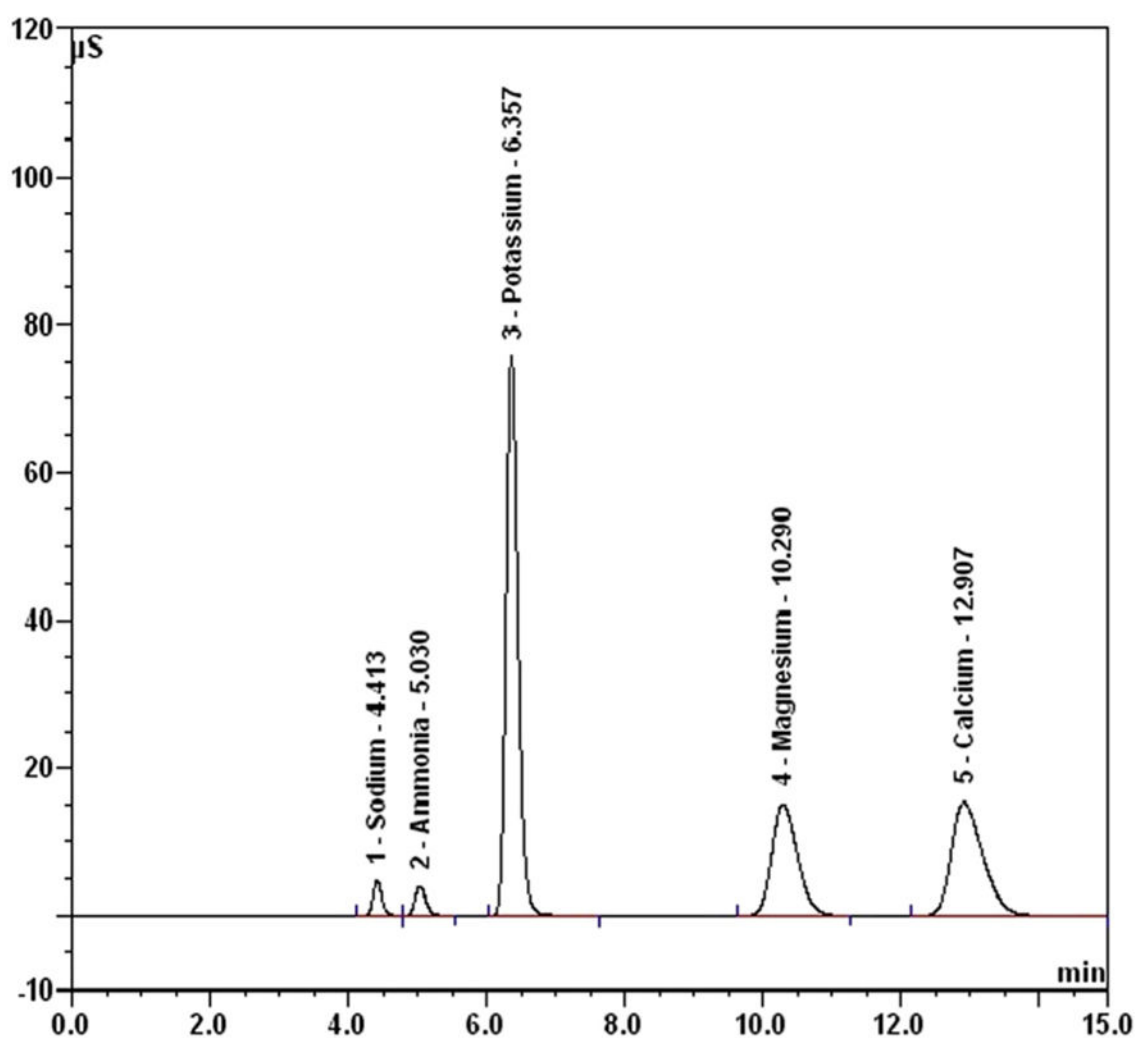


Fig. 2.
Decay rate for a high and low ammonia content cigarette upon opening of packs.

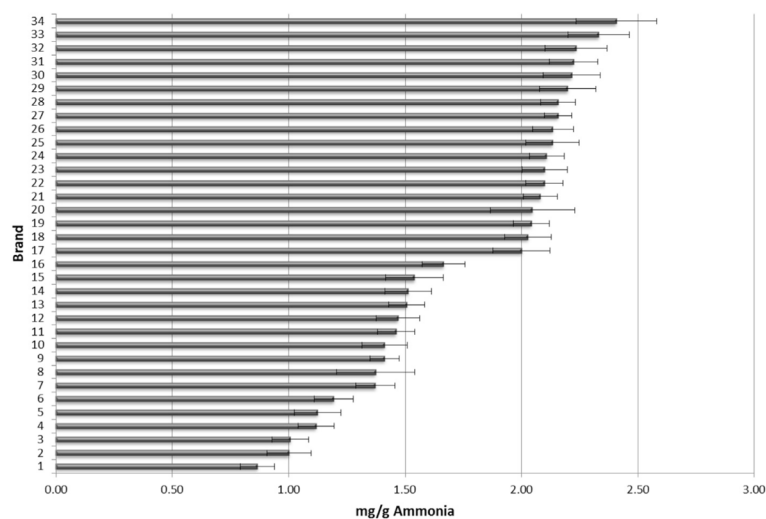


Fig. 3.
Average ammonia content across 34 domestic brands analyzed.

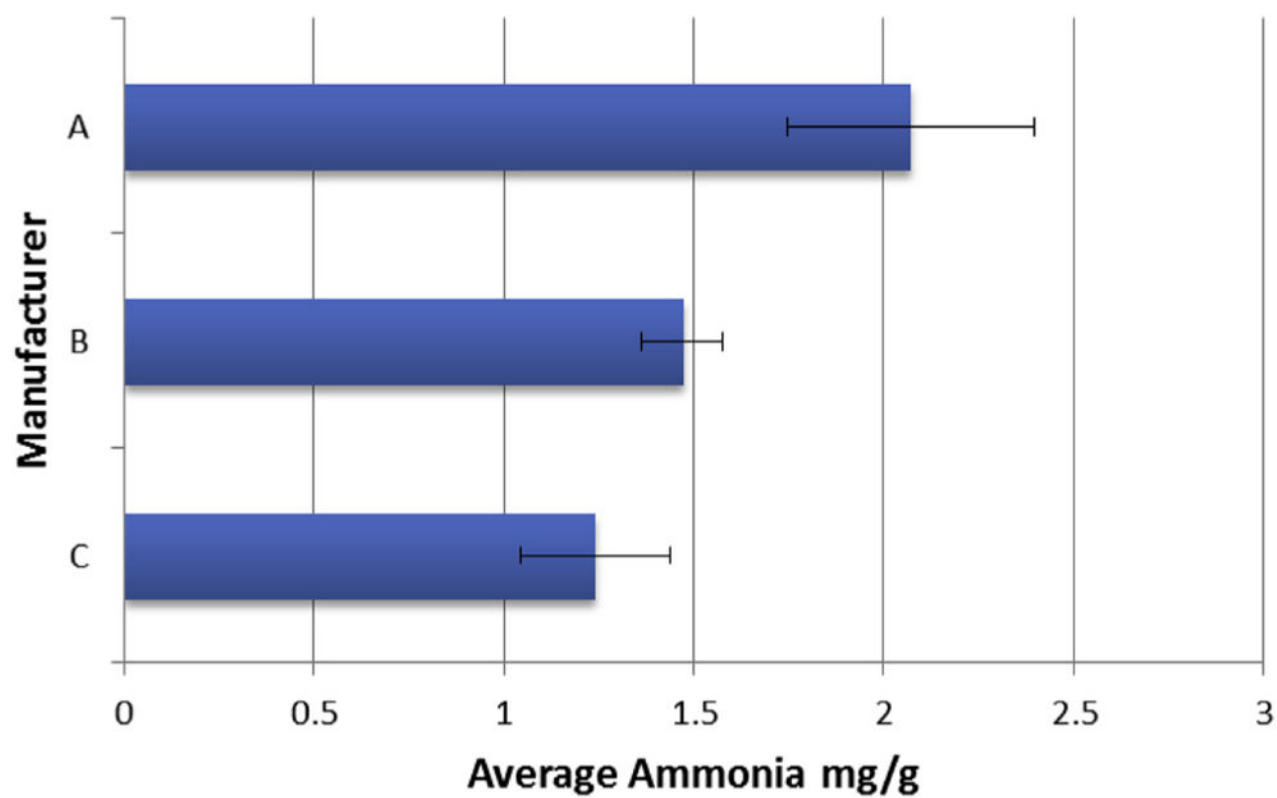


Fig. 4.
Average ammonia by manufacturer.

Table 1

Average ammonia content for different extraction solutions.

Extraction solution	DI water	0.025 N H ₂ SO ₄	5% acetic acid
Average ammonia (mg/g)	2.03 ± .17	1.97 ± .15	2.12 ± .06

Table 2

Comparison of 3R4F/1R4F average ammonia values.

Study	3R4F/1R4F values (mg/g)
Baker 2004 (HPLC) (Baker et al., 2004)	0.85 ± .16 (1R4F)
Counts 2005 (IC) (Counts et al., 2005)	0.83 ± .01 (1R4F)
Counts 2006 (Colorimetry) (Counts et al., 2006)	1.6 ± 0.05 (dry weight) (2R4F)
Our study (IC)	1.1 ± .14 (3R4F)